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Impact of predicted precipitation scenarios on multitrophic interactions

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Manuscripts

1 **Impact of predicted precipitation scenarios on multitrophic interactions.**

2

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4

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12

13 Running head: Drought/deluge influence trophic interactions

14 Summary

- 15 1. Predicted changes in the frequency and intensity of extreme rainfall events in
16 the UK have the potential to disrupt terrestrial ecosystem function. However,
17 responses of different trophic levels to these changes in rainfall patterns, and
18 the underlying mechanisms, are not well characterised.
- 19 2. This study aimed to investigate how changes in both the quantity and
20 frequency of rainfall events will affect the outcome of interactions between
21 plants, insect herbivores (above- and below- ground) and natural enemies.
- 22 3. *Hordeum vulgare* L. plants were grown in controlled conditions and in the
23 field, and subjected to three precipitation scenarios: ambient (based on a local
24 10 year average rainfall); continuous drought (40% reduction compared to
25 ambient); drought/ deluge (40% reduction compared to ambient at a reduced
26 frequency). The effects of these watering regimes and wireworm (*Agriotes*
27 *species*) root herbivory on the performance of the plants, aphid herbivores
28 above-ground (*Sitobion avenae*, *Metapolophium dirhodum* and
29 *Rhopalosiphum padi*), and natural enemies of aphids including ladybirds
30 (*Harmonia axyridis*) were assessed from measurements of plant growth,
31 insect abundance and mass, and assays of feeding behaviour.
- 32 4. Continuous drought decreased plant biomass, whereas reducing the
33 frequency of watering events did not affect plant biomass but did alter plant
34 chemical composition. In controlled conditions, continuous drought
35 ameliorated the negative impact of wireworms on plant biomass.
- 36 5. Compared to the ambient treatment, aphid mass was increased by 15% when
37 feeding on plants subjected to drought/ deluge; and ladybirds were 66%

38 heavier when feeding on these aphids but this did not affect ladybird prey
39 choice. In field conditions, wireworms feeding below-ground reduced the
40 number of shoot-feeding aphids under ambient and continuous drought
41 conditions but not under drought/ deluge.

42 6. Predicted changes in both the frequency and intensity of precipitation events
43 under climate change have the potential to limit plant growth, but reduce
44 wireworm herbivory, while simultaneously promoting above-ground aphid
45 numbers and mass, with these effects transferring to the third trophic level.
46 Understanding the effect of future changes in precipitation on species
47 interactions is critical for determining their potential impact on ecosystem
48 functioning and constructing accurate predictions under global change
49 scenarios.

50 **Keywords** *Agriotes*, climate change, extreme events, *Harmonia axyridis*, herbivory,
51 *Hordeum vulgare*, *Sitobion avenae*.

52 **Introduction**

53 Climate models predict that by 2080 there will be increased frequency and intensity
54 of drought and heavy rainfall events in the UK, with overall reductions of up to 40%
55 in summer precipitation volume (Murphy *et al.* 2009; Bouwer *et al.* 2014). Extreme
56 precipitation events are predicted to destabilise terrestrial ecosystems (Knapp *et al.*
57 2008) through alterations in resources, such as changes in plant growth and chemical
58 composition, and by disrupting interactions between plants and herbivores. This can
59 result in asynchrony between the development, behaviour and life cycles of different
60 trophic levels (Weltzin *et al.* 2003; Trotter, Cobb & Whitham 2008). Multi-trophic
61 interactions are critical in ecosystem structure and function (Hellmann *et al.* 2008),
62 and understanding the effects of future changes in precipitation on such interactions
63 is important for food security, pest management and constructing more accurate
64 predictions of global change impacts (van der Putten *et al.* 2004). Despite a large
65 amount of evidence predicting changes in precipitation patterns and their potential to
66 disrupt ecosystems, there is very little published research attempting to simulate
67 changes in the frequency as well as the intensity of rainfall events and test how this
68 will impact multi-trophic interactions (Weltzin *et al.* 2003; Facey *et al.* 2014).

69

70 Some plants can tolerate or adapt to water stress through a number of mechanisms
71 such as changes in resource allocation to growth and development (Blum 1996;
72 Chaves, Maroco & Pereira 2003) and osmotic adjustment including utilising sugars
73 as osmoprotectants to minimising oxidative damage (Chaves, Maroco & Pereira
74 2003; Barnabás, Jäger & Fehér 2008). Increased silicon (Si) uptake has also been
75 reported to improve the tolerance of plants to water stress by stimulating antioxidant

76 systems, immobilising antioxidants avoiding cellular damage by reactive oxidative
77 damage (Gong *et al.* 2005, 2008; Pei *et al.* 2010) and providing cellular structural
78 support to avoid lodging (Ma 2004; Cooke & Leishman 2011; Balakhnina *et al.*
79 2012). These changes in plant morphology, physiology and chemical composition
80 due to water stress can influence plant food quality for above- and below- ground
81 arthropod herbivores, which in turn can affect herbivore performance (Huberty &
82 Denno 2004; Chown, Sørensen & Terblanche 2011). To date, most current research
83 focuses on the impact of continuous drought or complete water withholding events
84 on insect herbivore populations, despite evidence to suggest that the severity of
85 drought events maybe an important determinant of the outcome for root and foliar
86 feeding herbivores (Mody, Eichenberger & Dorn 2009; Jamieson *et al.* 2012; Tariq
87 *et al.* 2012; Rosenblatt & Schmitz 2014). For example, for sap feeding insects such
88 as aphids, ‘pulsed water stress’ arising from frequent drought and recovery events is
89 thought to be beneficial due to increased foliar nitrogen availability and periods of
90 turgor recovery (Larsson 1989; Huberty & Denno 2004; Mody *et al.* 2009). The
91 timing of these extreme rainfall events within the growing seasons is particularly
92 important (Griffin & Hoffmann 2011; de San Celedonio, Abeledo & Miralles 2014).
93 However, there is currently a lack of research investigating how changes in the
94 frequency of rainfall events throughout the growing season would affect crop growth
95 and whether these effects transfer to higher trophic levels.

96

97 Water stress can also affect the direction and intensity of interactions between above-
98 and below- ground insect herbivores through changes in root herbivore behaviour
99 and host plant growth and chemical composition (Staley *et al.* 2007). The severity
100 and number of days of a drought treatment or level of reduction in soil moisture has

101 also been reported to influence the abundance and vertical distribution of below-
102 ground insect herbivores (Lees 1943a; Briones, Ineson & Pearce 1997; Sinka, Jones
103 & Hartley 2007), potentially impacting their feeding behaviour. Root herbivores
104 themselves can influence host plant growth, development and chemical composition
105 (Johnson, Erb & Hartley 2016) and can also increase the severity of a drought event
106 to plants due to the removal of roots, thereby altering the quality of the plant as a
107 food source for the other organisms feeding on the same plant (Bezemer & van Dam
108 2005; Tariq *et al.* 2013a). Therefore, any changes in below-ground herbivore feeding
109 intensity due to changes in soil moisture availability have the potential to also impact
110 above-ground herbivores. The impact of herbivory below-ground on above-ground
111 herbivores has specific importance as there is evidence to suggest that above-,
112 below-ground interactions can affect the third trophic level (Barnett & Johnson
113 2013; Johnson *et al.* 2013), with potential to influence ecosystem functions
114 associated with the wider insect community.

115

116 Effects of water stress have also been previously reported to transfer into higher
117 trophic levels (Johnson *et al.* 2011) influencing the fitness and abundance of natural
118 enemies of insect herbivores as a result of changes in prey quality, mediated by
119 changes in the host plant (Ledger *et al.* 2012; McCluney *et al.* 2012). However, the
120 very few studies investigating the impact on the third trophic level focus on
121 parasitoids (e.g. Johnson *et al.* 2011; Aslam, Johnson & Karley 2013; Tariq *et al.*
122 2013b) and do not consider how this will influence insect herbivore quality as prey
123 for insect predators. Moreover, the majority of research is conducted in controlled
124 environment systems. Although controlled environment experiments provide
125 fundamental understanding of species interactions within complex systems,

126 conditions in these environments might not reflect those of the field environment
127 (Hughes 1959) which differ in rates of soil drying, air flow, radiation, temperature
128 and soil structure. Very few studies use a combination of controlled environment
129 conditions and field conditions to determine how relevant their findings are to the
130 natural environment. Research measuring the impact of realistic rainfall patterns in
131 field conditions and determining if studies conducted in controlled environments
132 produce reliable results compared to field conditions is particularly lacking.
133 Therefore, here we aim to increase fundamental understanding of how changes in
134 rainfall quantity and pattern can interact with different herbivore guilds and transfer
135 to a third trophic level. To our knowledge, this is the first study to achieve this
136 trophic complexity in both field and controlled conditions.

137

138 This study investigates the effect of predicted changes in precipitation (in both
139 rainfall quantity and frequency) using realistic water stress scenarios on plant-
140 herbivore interactions above- and below-ground. We used root- (wireworm *Agriotes*
141 spp.) and shoot-feeding herbivores (aphid *Sitobion avenae* F., *Metopolophium*
142 *dirhodum* Walker and *Rhopalosiphum padi* L.) commonly found attacking barley as
143 a model system (Johnson, Hawes & Karley 2009), and examined the effect of water
144 stress on the performance of common natural enemies of this herbivore, including
145 the invasive Harlequin ladybird (*Harmonia axyridis*) (Majerus, Strawson & Roy
146 2006) and parasitoid wasps such as *Aphidius ervi*. Experiments were conducted in
147 controlled environment conditions and in field mesocosms, to assess if plant and
148 insect herbivore responses are consistent between different experimental conditions.
149 It was hypothesised that (i) continuous drought and drought/ deluge would reduce
150 barley growth but positively affect aphid development and fecundity, with drought/

151 deluge having larger effects on both plants and aphids; (ii) wireworm root feeding
152 would positively affect performance of aphids above-ground, but reduced water
153 availability would mitigate this interaction and (iii) increased aphid performance
154 (due to changes in water availability or wireworm herbivory) would benefit the
155 performance of natural enemies.

156

157 **Materials and Methods**

158 *Plant growth conditions*

159 Two separate experiments were performed, one with plants grown in pots in
160 controlled environment conditions and the other with plants grown in pots positioned
161 in a field situated at the James Hutton Institute, Dundee, UK. For both experiments
162 *H. vulgare* spring barley cultivar Optic (seeds supplied by The James Hutton
163 Institute, Dundee, UK) plants were grown in pots (see below for details) filled with
164 dried, sieved (10 mm x 10 mm aperture sieve) topsoil (A1 Plant, Elvington, UK)
165 mixed in a 3:1 ratio with washed sharp horticultural sand (Keith Singletons,
166 Egremont, UK) to give a sandy loam soil substrate. Prior to the experiment all pots
167 were watered with deionised water from the top of the pot to ensure soil water
168 content reached 50% of total water holding capacity and soil water content was
169 maintained at 50% total water holding capacity for the first two weeks to ensure
170 seedling establishment (see Appendix S1 in Supporting Information).

171

172 Three different watering regimes were applied to the plants:

173 (i) Ambient = a quantity of water added based on 10 year weekly average
 174 rainfall at the James Hutton Institute, Invergowrie, Scotland. Half of the
 175 weekly average was provided twice per week;

176 (ii) Drought = a 40% reduction in the quantity of water added, also provided
 177 twice per week;

178 (iii) Drought/deluge = a 40% reduction in the quantity of water added with
 179 severe reduced watering frequency, provided once per fortnight.

180 Quantities of water added to each pot were calculated based on pot surface area (see
 181 Appendix S1). These different watering regimes allowed comparison of the effects
 182 of reductions in rainfall quantity (a 40% reduction compared to ambient) and
 183 reductions in rainfall frequency under the 40% reduction regime (water provided
 184 once per fortnight compared with twice per week).

185

186 For plants assigned the below-ground herbivory treatment, two weeks (controlled
 187 environment experiment) or three weeks (field experiment; to account for slower
 188 plant development in the field) after sowing, three wireworms (a mixture of *Agriotes*
 189 spp. L. *Coleoptera: Elateridae*: 60% *A. lineatus* and 40% *A. obscurus* sourced from
 190 Praktijkonderzoek Plant and Omgeving / Applied Plant Research, Wageningen, UR)
 191 were buried c. 5cm from the soil surface in three different locations within the pot. A
 192 similar level of soil disturbance was imposed on pots that were not assigned
 193 wireworm treatment.

194

195 At harvest, plant ear, leaf, stem and root fractions were collected separately, weighed
 196 for fresh mass, dried at 70 °C for c. five days and re-weighed for dry mass (g dry

197 mass DM). One exception to this was the leaf fraction of plants grown in the
198 controlled environment experiment; prior to weighing, two green leaves were
199 removed for amino acid analysis as described below. The dry mass of these leaves
200 was calculated based on the fresh mass: dry mass ratio of the rest of the leaf fraction,
201 which was processed for oven drying in the same way as the other plant fractions.
202 Root: mass ratio and water content (g) of the plant material was calculated (see
203 Appendix S1).

204

205 *Controlled environment experiment*

206 The experiment comprised a randomised block design to account for spatial variation
207 within the controlled environment space. The experiment was conducted in three
208 growth rooms, with each room divided equally into three spatial blocks (nine blocks
209 in total). Within each block (12 plants), each combination of watering regime (three
210 levels; see above) and herbivory (four levels: control (no herbivore), above- (aphids),
211 below- (wireworms) ground herbivore or both) were assigned at random to each pot
212 location. This resulted in one replicate plant per block of each watering regime x
213 herbivory combination, with nine replicate blocks giving a total of 108 plants. Plants
214 were grown individually in 2.4 L pots (see Appendix S1) maintained at 16 h daylight
215 (average light intensity across the three rooms was $210.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ mean ± 1.80
216 standard error); $23^\circ\text{C} \pm 0.33$ / $19^\circ\text{C} \pm 0.21$, day / night. Five weeks after sowing,
217 stomatal conductance of the barley plants was measured on six replicate plants (six
218 blocks) for each watering regime and herbivory treatment using a Porometer (AP4
219 Leaf Porometer, Delta-T Devices, Cambridge UK). Porometer readings were taken
220 between 0900 h and 1000 h (BST).

221

222 Three aphid (*S. avenae*) nymphs (born on the plant see Appendix S1) were

223 monitored daily for c. four weeks to record aphid survival, the date of first

224 reproduction and number of offspring produced. Fecundity was calculated as the

225 number of offspring produced in the same number of days it took for the adult to

226 mature. Offspring were collected every two days and transferred to a second cage

227 (cage 2) clipped onto on the same plant. After four weeks of aphid herbivory, all

228 aphids were individually counted, removed from the plants and weighed, before

229 using in the ladybird performance assays (see below).

230

231 Ladybird responses to the water treatments were assessed using a combination of

232 feeding and choice assays: one second instar Harlequin ladybird (*H. axyridis*) was

233 weighed, and then presented with three pre-weighed apterous adult aphids and three

234 first instar nymphs (collected from cage 2; see above), in a Petri-dish (90 mm,

235 Sterilin Ltd, Mid Glamorgan, UK). Assays were conducted over 24 h in a controlled

236 environment room (12 h daylight; c. 20 °C), after which the ladybird was removed,

237 re-weighed and mass gain calculated (11 to 13 replicates). For the ladybird prey

238 choice assay: three live apterous adult aphids, one from a plant treated with each

239 watering regime within the same block, were collected and positioned randomly in a

240 90 mm Petri-dish (Sterilin Ltd, Mid Glamorgan, UK) and equidistant from the dish

241 centre. The ventral surface of each aphid was secured to the base of the dish with a

242 small (c. 3 mm x 4 mm) piece of double sided sticky tape, leaving their legs free to

243 defend from predator attack, a common form of defence for aphids (Roy *et al.* 2013).

244 One harlequin ladybird larva (third or fourth instar) was placed in the middle of the

245 arena. The ladybird larvae were monitored and the first aphid to be consumed was
246 recorded.

247

248 Barley plants in each experimental block were harvested seven weeks after sowing,
249 at Zadoks growth stage 40 (Zadoks, Chang & Konzak 1974). Elemental analysis was
250 conducted on dried milled green leaf material (*c.* four oven-dried green leaves per
251 plant) (see Appendix S1). Si concentration (% dry mass) was determined using a
252 commercial P-XRF instrument (Niton XL3t900 GOLDD analyser: Thermo
253 Scientific Winchester, UK) held in a test stand (SmartStand, Thermo Scientific,
254 Winchester, UK) (Reidinger *et al.* 2012). The carbon (C) and nitrogen (N)
255 concentrations of leaf (% dry mass) were determined by flash combustion and
256 chromatographic separation of ~1.5 mg milled leaf using an elemental analyser
257 (Elemental combustion system 4010 CHNS-O Analyser, Costech Analytical
258 Technologies, Inc., Milan, Italy), calibrated against a standard (C₂₆H₂₆N₂O₂S).

259

260 Total amino acids were extracted (see Appendix S1) from 25 mg of freeze-dried,
261 milled green leaf material using 1 mL solution of 49% methanol, 49% milli-Q water
262 and 2% glacial acetic acid (adapted from Matsuda *et al.* 2005; Noctor *et al.* 2007)
263 and were analysed by HPLC to quantify amino acid composition. Amino acids were
264 separated by reverse-phase HPLC and quantified as described by (Johnson *et al.*
265 2009).

266

267 *Field experiment*

268 Nine spring barley plants were grown in 15 L pots (25.5 cm × 25.5 cm × 25.5 cm)
269 lined with plastic sheeting. Pots were wrapped in insulating material (Thermawrap
270 loft insulation, 400 mm x 5 m, B&Q, UK) and covered in Fine Mesh Garden
271 Protection Net (2 cm diameter netting, B&Q, UK) which was suspended c. 50 cm
272 above the pot and draped down the sides of the pots to prevent small mammalian
273 herbivory but to allow arthropod access to the plants. Theta probes (Delta-T ML2,
274 connected to a DL6 data logger, and downloaded using DeltaLINK software, Delta-
275 T, Cambridge, UK) were buried horizontally 10 cm from the soil surface in all six
276 pots under one rain exclusion shelter (one block) to measure soil moisture every min
277 throughout the experimental period. All plants were grown under rain exclusion
278 shelters including the ambient treatment. A Met station positioned in the same field
279 provided meteorological data for the experiment. Maximum air temperatures were
280 on average $18.4^{\circ}\text{C} \pm 0.3$ with minimum temperatures averaging $9.4^{\circ}\text{C} \pm 0.3$.

281

282 The experiment comprised a randomized block design with five blocks (rain
283 exclusion shelters). Within each block, watering regime and root herbivory were
284 assigned at random to each pot, with one replicate per block of each watering regime
285 × herbivory combination (six pots under each rainshelter).

286

287 Plants were open to natural establishment of above-ground herbivores and natural
288 predators. Three barley plants in each pot selected at random and identified using a
289 small piece of cotton tied very loosely around the main stem were used for routine
290 monitoring of insect herbivore and natural enemy abundance. Total numbers of
291 aphids (*Sitobion avenae*, *Metapolophium dirhodum* and *Rhopalosiphum padi*) on

292 these plants were recorded weekly throughout the growing period as well as the
293 number of mummified aphids (parasitised aphids) to assess the combination of
294 acceptance of aphids for oviposition and the suitability of aphids for parasitoid
295 survival. Barley plants in each experimental block were harvested 10 weeks after
296 sowing, by which time the ear on the main stem of all plants had reached Zadok's
297 growth stage 71 (Zadoks *et al.* 1974). Material from all nine plants within the pot
298 was pooled. At harvest, all aphids were collected from all the plants in each pot and
299 counted, then transferred to 1 mL Eppendorf tubes, flash frozen in liquid nitrogen
300 and stored at -20 °C. Frozen aphids were then freeze-dried and re-weighed, and
301 individual aphid mass calculated by dividing total aphid mass per pot by the number
302 of aphids collected.

303

304 *Statistical analysis*

305 Statistical analyses were performed in R (version 3.0.2) to test the main and
306 interactive effects of watering regime, and above- and/ or below- ground herbivory
307 treatment on the measured variables. Data were checked for normality and
308 homogeneity of variance by plotting Q-Q plots and residuals vs fitted values.
309 Significance was set at $P < 0.05$ for all analyses. To meet the assumptions of the linear
310 mixed effect model, proportion data were arcsine square root transformed (root:
311 mass, Si data) and controlled environment experiment total plant biomass data were
312 squared, amino acid data were transformed using natural log and aphid biomass data
313 were square root transformed. Linear mixed-effects models (lme from package nlme)
314 (Pinheiro *et al.* 2014) were used to analyse continuous data with block included in
315 the model as a random term. Generalised linear mixed-effect models (glmer from
316 package lme4) (Bates *et al.* 2014) were used to analyse count data. Models were

317 compared using AIC values and analysis of variance (ANOVA) for stepwise
318 elimination of non-significant terms to find the minimum adequate model (Crawley
319 2007) and the final models were then analysed using ‘anova’ (F statistic) or ‘Anova’
320 (‘car’ package (Fox *et al.* 2014) χ^2 statistic). Multiple comparison tests were
321 performed using ‘glht’ in multcomp package with *post-hoc* Tukey contrasts
322 (Hothorn *et al.* 2014). For controlled environment experimental data, all measured
323 growth parameters were assessed on nine replicates (plants) for each watering
324 regime and herbivory treatment apart from ambient watered plants with no herbivory
325 which was assessed on eight plants due to a plant fatality. Leaf Si was assessed on
326 seven replicates and aphid mass (g FM) was assessed on 14 to 16 replicates. The
327 field experiment was assessed on five replicates for each watering regime and
328 herbivory treatment.

329

330 For controlled environment experiment, aphid mass gain was analysed using linear
331 mixed effect models to test the main effects of watering regime, wireworm treatment
332 with block and clip cage included as a random term. Ladybird mass gain was also
333 analysed using linear mixed effect models to test the main effects of watering
334 regime, wireworm treatment with block and a category of aphid mass (FM) included
335 as a random term. Ladybird prey choice was analysed using generalised linear mixed
336 effects model (*glmer*) (Bates *et al.* 2014) to test the main effects of watering regime,
337 wireworm treatment, and ladybird and aphid mass (FM) with arena included as a
338 random term. Ladybird mass gain was assessed on five to seven replicates and
339 ladybird prey choice was assessed on four to eight replicates.

340

341 Amino acid concentrations were converted to $\mu\text{mol/g}$ leaf dry mass prior to analysis.
342 Variation in plant amino acid concentration ($\mu\text{mol/g}$) and composition was explored
343 by principal components analysis (PCA) performed on Minitab 17. Pearson product-
344 moment correlation coefficient was used to measure if there was a linear correlation
345 between aphid mass and N or total amino acids.

346

347 **Results**

348 *Impact of the different watering regimes on wireworm herbivory and plant growth*
349 *and development.*

350 Regardless of herbivory treatment, a 40% reduction in water quantity significantly
351 reduced total plant biomass, whereas reducing the frequency of watering events had
352 no effect on total plant biomass (Fig. 1). In controlled environment conditions, total
353 plant biomass was significantly reduced by wireworm herbivory under ambient
354 watering regime (*Post-hoc* Tukey test $=P<0.001$) (Fig. 1a), but wireworms had no
355 effect on total plant biomass under continuous drought and drought/ deluge watering
356 regime. In contrast, wireworms had no effect on plant biomass in the field
357 experiment (Fig. 1b). There was also no effect of wireworm herbivory ($F_{1,20}=0.52$,
358 $P=0.48$) or watering regime ($F_{2,20}=2.76$, $P=0.087$) on root: mass ratio. There was no
359 effect of aphid herbivory recorded on plant biomass ($F_{1,92}=1.301$, $P=0.257$).

360

361 In the field, soil moisture in the continuous drought treatment was lower than that in
362 the ambient treatment (see Figure S1 in Supporting Information). Soil in the drought/
363 deluge treatment showed a large increase in moisture immediately after a watering
364 event which slowly declined over the next two weeks until the next watering event.

365

366 *Aphid survival, development and reproduction*

367 In the controlled environment, the number of days to aphid reproduction, aphid
 368 fecundity and aphid survival were not affected by either wireworms or watering
 369 regime or the interaction between these factors (see Table S1 in Supporting
 370 Information). Individual mass of aphids collected from drought/ deluge treated plants
 371 was significantly heavier than those collected from ambient treated plants (Fig. 2a)
 372 but was unaffected by wireworm herbivory ($F_{1,36}=0.019$, $P=0.89$).

373

374 In the field, wireworms caused a significant reduction in total number of aphids on
 375 drought treated plants five and six weeks after sowing and on ambient watered plants
 376 six weeks after sowing (Fig. 3). Total aphid biomass (g DW) was unaffected by
 377 changes in the watering regime ($F_{2,23}=1.78$, $P=0.19$), or by wireworm herbivory
 378 ($F_{2,23}=0.79$, $P=0.47$).

379

380 *Third trophic level*

381 Ladybird larvae in the controlled environment experiment gained significantly more
 382 mass when feeding on aphids collected from plants under drought/ deluge watering
 383 regime compared to when feeding on aphids collected from ambient watered plants
 384 (Fig. 2b). Wireworm herbivory of the aphid's host plant had no effect on ladybird
 385 mass gain ($F_{1,11}=0.241$, $P=0.63$). Ladybird larvae choice was unaffected by the
 386 watering regimes ($\chi^2=1.379$, $df=2$, $P=0.502$), or by wireworm herbivory ($\chi^2=0.000$,
 387 $df=1$, $P=0.995$).

388

389 There were very few natural enemies recorded throughout the field experiment. Total
390 number of mummified aphids was unaffected by the watering regime ($\chi^2=2.519$,
391 $df=2$, $P>0.05$) and wireworm herbivory ($\chi^2=0.850$, $df=1$, $P>0.05$).

392

393 *Plant chemical composition*

394 In the controlled environment, tissue water content (g) was significantly greater in
395 ambient watered plants compared to drought and drought/ deluge ($F_{2,94}=67.841$,
396 $P<0.0001$) and smaller in plants subjected to wireworm herbivory ($F_{1,95}=6.474$,
397 $P=0.0126$) (see Figure S2a in Supporting Information). Aphids had no effect on plant
398 water content ($F_{1,94}=0.655$, $P=0.4203$). Drought and drought/ deluge treated plants
399 had a significantly lower stomatal conductance compared to ambient watered plants
400 ($F_{2,62}=29.064$, $P<0.001$; *Post-hoc* Tukey contrasts $P<0.001$) (see Figure S2b).
401 Wireworms ($F_{1,62}=0.086$, $P=0.770$) and aphids ($F_{1,62}=1.097$, $P=0.299$) were found to
402 have no impact on stomatal conductance. Plants grown under ambient watering
403 regime had the highest leaf Si concentration compared to drought/ deluge and
404 drought treated plants, with drought treated plants containing the lowest leaf Si
405 concentrations (Fig. 4a). Neither wireworm ($F_{1,65}=0.009$, $P=0.93$) nor aphid
406 ($F_{1,65}=0.319$, $P=0.57$) herbivory had any effect on leaf Si concentration.

407

408 Regardless of herbivory treatment, plants grown under drought and drought/ deluge
409 watering regimes had a higher leaf N concentration than ambient watered plants.
410 Wireworm herbivory significantly increased leaf N concentration (Fig. 4b). Aphids
411 ($F_{1,66}=0.204$, $P=0.6532$) had no effect on leaf N concentration. Drought/ deluge
412 plants had higher concentration of amino acids compared to ambient treated plants

413 with the concentration of amino acids in drought treated plants intermediate (but not
 414 significantly different) from ambient or drought/ deluge treated plants (Fig. 4c).
 415 Drought treated plants had a significantly higher concentration of essential amino
 416 acids compared to plants under the ambient watering regime ($F_{2,88}=4.701$, $P<0.05$;
 417 *Post-hoc* Tukey test $P<0.01$). Foliar essential amino acid concentrations were
 418 significantly increased by aphid herbivory ($F_{1,88}=5.436$, $P=0.022$), but wireworms
 419 had no effect on essential amino acids ($F_{1,88}=2.245$, $P=0.138$). Total amino acids
 420 comprised 17% essential amino acids under drought conditions compared to 14%
 421 essential amino acids under ambient watering. Therefore the drought treatment
 422 increased the proportion of essential amino acids ($F_{2,81}=8.051$, $P<0.001$). Visual
 423 exploration of the amino acid data by PCA revealed that PC1 (accounting for 68.8%
 424 of the variation in the data set) separated glutamate, tryptophan and methionine from
 425 all other amino acids. Tryptophan, glutamine and glutamate were separated along
 426 PC2 (22% of the variation; Fig. 5a). A plot of the score values indicated that these
 427 two axes separated ambient plants from the other watering regimes suggesting that
 428 quantity of water has a greater effect than changes in the frequency of watering
 429 events on amino acid composition (Fig. 5b). There was no correlation between aphid
 430 mass and total amino acid concentration ($R=-0.0036$, $P=1.00$) or leaf N
 431 concentration ($R=0.304$, $P=0.060$).

432

433 Discussion

434 This study shows that predicted changes in both the frequency and intensity of
 435 precipitation events can have significant impacts on above-ground multi-trophic
 436 interactions, reducing plant growth but also reducing the impact of wireworm

437 herbivory below-ground whilst potentially increasing aphid and ladybird
438 performance above-ground. Plant responses to the watering regimes were similar in
439 the controlled environment and the field mesocosm experiment but insect herbivore
440 responses differed between the two experiments.

441

442 *The impact of changes in precipitation on plant growth and chemical composition*

443 Plant growth was reduced under predicted rainfall scenarios, but changes in the
444 frequency of rainfall events had very little impact on plant biomass. This was
445 surprising as it was originally predicted that changes from dry conditions to flooding
446 would increase the level of stress for plants. However, soil moisture measured by the
447 theta probes buried half way down the soil profile demonstrated that although water
448 quantity was reduced by 40% under the drought/ deluge watering regime, reducing
449 the watering frequency resulting in deluge events caused soil moisture in the deeper
450 soil profile to remain wetter than pots watered more frequently. This suggests that an
451 extreme rainfall event during periods of drought could facilitate water penetration to
452 a deeper soil profile leading to the bulk soil remaining wetter for longer, benefiting a
453 deeper rooting zone and enabling water uptake during periods of drought (Heisler-
454 White *et al.* 2009). In comparison, regular, lighter precipitation events may only
455 penetrate the topsoil which is more exposed, and soil moisture is more likely to be
456 lost due to evaporation. However, barley plants have been recorded to root deeper
457 than 25 cm (the depth of the pot) (Lampurlanés, Angás & Cantero-Martínez 2001),
458 which would potentially enable these plants to have access to water stores deeper in
459 the soil profile in a field system. Similar soil moisture patterns were reported by Fry
460 *et al.* (2014) where a drought/ deluge treatment in a grassland system also resulted in

461 the soil remaining wetter for a longer duration, but this treatment was reported to
462 reduce plant biomass and species richness.

463

464 Drought/ deluge treated plants exhibited lower Si concentrations and tissue water but
465 higher leaf N and amino acid concentrations compared to ambient watered plants.

466 The accumulation of free amino acids and nitrogen-containing osmoprotectants is
467 often recorded in watered stressed plants (both drought and over-watering
468 conditions), and is thought to be a mechanism to offset low osmotic pressure
469 (Huberty & Denno 2004; Khan, Ulrichs & Mewis 2010). The relative changes in
470 amino acids tryptophan, methionine, glutamine and glutamate explain a large
471 proportion of the impacts of drought on total amino acids. Tryptophan particularly is
472 classed as an essential amino acid and can play a role in reducing the effects of
473 drought in maize when applied as a treatment (Rao *et al.* 2012) and has been found
474 to increase in drought stress wheat plants (Bowne *et al.* 2012). Silicon is mainly
475 taken up by plants passively through aquaporin-type transporters in the roots, so
476 reductions in transpiration rates under drought conditions may explain the reductions
477 in observed leaf Si (Ma & Yamaji 2006). However, despite receiving the same
478 quantity of water over the growing period, reducing the frequency of watering events
479 significantly increased leaf Si concentrations. Therefore, changing the frequency of
480 rainfall events could have increased the ability of the plant to take up Si, possibly
481 due to the deeper soil profile remaining wetter for longer affecting plant transpiration
482 rates and thus Si uptake (Hartley *et al.* 2015). In grasses, Si plays an important role
483 in plant defence against insect and mammalian herbivores (Massey, Ennos & Hartley
484 2006; Guntzer, Keller & Meunier 2012). Therefore, changes in leaf Si concentration
485 under future predicted precipitation regimes could influence the resistance of barley

486 to crop pests. Silicon levels were not found to affect aphid performance in this study,
487 supporting some previous evidence to suggest that Si physical defence may be more
488 effective against chewing insects rather than phloem feeders (Massey *et al.* 2006;
489 Reynolds, Keeping & Meyer 2009; Reynolds *et al.* 2016).

490

491 *The impact of changes in precipitation on aphids*

492 Under future precipitation patterns, aphid biomass was increased in the controlled
493 environment experiment. In the controlled environment experiment, heavier aphids
494 were collected from plants watered less frequently under the 40% reduction regime
495 most likely due to changes in plant nitrogen and amino acid concentration (White
496 1984). Insects cannot synthesize amino acids and research demonstrates that one of
497 the functions of the aphid obligate bacterial endosymbiont *Buchnera aphidicola* is to
498 synthesize tryptophan (Rouhbakhsh *et al.* 1996), confirmed by the fact that aphids
499 treated with antibiotic to disrupt the symbiosis exhibit high nymph mortality when
500 feeding on a synthetic diet lacking tryptophan (Douglas & Prosser 1992). It is
501 particularly interesting to note that drought had a large impact on the amino acid
502 tryptophan in the controlled environment experiment reported here. However, there
503 was no significant correlation found between aphid mass and nitrogen or amino acid
504 concentration which suggests that there were other factors, such as changes in turgor
505 pressure, influencing the impact of changes in N and amino acid availability on
506 aphid performance (Huberty & Denno 2004; Mody *et al.* 2009).

507

508 Insect body size has been reported to correlate with insect performance and fecundity
509 (Honek 1993), but in this study the number of offspring produced was unaffected by

510 the watering regime of the adult aphids' host plant. This is in contrast with the
511 results reported by Tariq *et al.* (2012), where generalist and specialist aphid
512 fecundity was highest on medium drought stressed plants compared to pulsed water
513 stress. In the field experiment however, despite large differences in plant biomass,
514 there was no effect of the different watering regimes on the abundance or mass of
515 aphids, suggesting that variable conditions in the field such as changes in
516 temperature during the experiment could have larger impacts on aphid survival,
517 abundance and fecundity (Bale *et al.* 2002; Nelson, Bjørnstad & Yamanaka 2013)
518 masking any effects of changes in precipitation.

519

520 *The impact of below-ground wireworm root feeding on above- ground aphid*
521 *performance*

522 Wireworms reduced aphid abundance on ambient and drought treated plants during
523 early plant development in the field experiment. Wireworms could have reduced the
524 number of aphids through a number of different mechanisms such as altering the
525 attractiveness of the host plant, increasing concentrations of defence compounds and/
526 or reducing the nutritional quality of the host plant (Bezemer & van Dam 2005;
527 Johnson *et al.* 2013). This is in contrast to previous published results which report
528 that below-ground herbivory positively affects above-ground herbivory (Johnson *et*
529 *al.* 2012). However, in the controlled environment there were no above- and below-
530 ground interactions recorded, despite the effect of wireworms on plant growth and
531 chemical composition. Plant development is clearly important in the interaction
532 between above- and below- ground herbivory, as the effect of wireworms on the
533 number of aphids in the field experiment was transient, only measured during early
534 plant development. In the field experiment, plant growth and development at harvest

535 was unaffected by wireworm herbivory; the low density of wireworms in each large
536 pot may have been insufficient to cause significant damage to fast growing, well
537 established, matured plants. Older plants are not as severely affected by wireworms
538 and plants in natural grasslands are often attacked by several wireworms at once
539 (Lees 1943b; Parker 1996; Parker & Howard 2001), so might be expected to be
540 resilient to the low experimental densities. Therefore contrasting results between the
541 two experiments may be due to differences in plant age and differences between
542 controlled environment condition and field environment which differ in rates of soil
543 drying, air flow, radiation, temperature and soil structure (Hughes 1959). Previous
544 published studies have also reported that the interaction between above- and below-
545 ground insect herbivores can change throughout the growing period, and that the
546 plant and insect species, as well as the developmental stage and feeding guild of the
547 insect herbivore, can influence the interactions between above- and below- ground
548 insect herbivores (Poveda *et al.* 2005; Johnson *et al.* 2012, 2013; Barnett & Johnson
549 2013).

550

551 *Do the effects of changes in precipitation impact the third trophic level?*

552 Changes in water availability and below-ground herbivory were found to affect the
553 potential fitness of a predator mediated by the plant and herbivore. To date, very
554 few studies have investigated the impact of water stress on multi-trophic interactions
555 encompassing above- and below- ground interactions particularly in agroecosystems
556 (Hentley & Wade 2017) despite reports that higher trophic levels may be more
557 sensitive to changes in climate (Voigt *et al.* 2003). Ladybirds were reported here to
558 have a greater increase in mass when feeding on aphids collected from plants grown
559 under reduced watering frequency. The increase in mass gain is likely to have been

560 due to differences in aphid mass. However, water regime may have also affected
561 handling time (e.g. larger aphids are better able to defend themselves), which might
562 have influenced final ladybird mass. Insect mass is often correlated with increased
563 insect fecundity and performance (Awmack & Leather 2002), therefore ladybird
564 fitness maybe higher when feeding on aphids from plants grown under future
565 predicted rainfall patterns. However, ladybird choice of aphid prey was unaffected
566 by the different watering regimes, despite the increase in mass when feeding on
567 aphids collected from ambient treated plants. This has consequences for the
568 performance of predators in this system and could impact their effectiveness as
569 natural enemies of crop pests. Previous research also reports that predator choice
570 does not follow optimal prey diet (Sih & Christensen 2001). This could influence
571 ladybird success under future changes in precipitation (Hassel & Southwood 1978;
572 Mayhew 2001). Nitrogen availability often limits insect growth (Mattson 1980)
573 therefore the reduction in mass gain of the ladybirds is potentially due to changes in
574 nitrogen (e.g. amino acid) concentration and composition of their prey mediated by
575 the host plant. Predators who feed on chewing herbivores maybe further affected by
576 changes in precipitation patterns mediated by the host plant due to potentially larger
577 reductions in chewing herbivore quality and quantity as a food source because of
578 increased leaf Si concentration of the host plant (Massey & Hartley 2009).

579

580 Parasitoid wasps were the most common natural enemy found on the plants
581 throughout the field experiment, but there was no significant effect of the watering
582 regimes on the numbers of mummified aphids. In contrast, published studies report
583 significant effects of changes in plant water status on the population of mummified
584 aphids (Aslam *et al.* 2013; Tariq *et al.* 2013b). However, these studies were

585 conducted in a controlled environment glasshouse. A field system may differ to
586 controlled environment due to varying temperatures, mummified aphid predation
587 and/or hyper-parasitism, which could influence mummified aphid abundance.

588

589 *Conclusions*

590 Barley plant growth was reduced under continuous drought whereas changes in the
591 frequency of precipitation events did not affect plant growth. However, changes in
592 the frequency of watering events removed the effects of wireworm herbivory on
593 plant growth and aphid abundance suggesting that the effect of wireworm herbivory
594 on this ecosystem will be reduced under future precipitation patterns. The drought/
595 deluge watering regime also caused significant changes in plant chemical
596 composition, as well as increased aphid mass and the mass of the ladybirds feeding
597 on these aphids. This study provides the first evidence that predicted changes in the
598 frequency as well as the intensity of rainfall events can affect plant growth and
599 chemical composition significantly as well as above- and below- ground insect
600 herbivores and their interactions, with these effects transferring to a third trophic
601 level, insect predators. Future research investigating the impact of predicted changes
602 in precipitation needs to consider changes in both the frequency and intensity of
603 precipitation events, as well as the experimental setting. These factors were shown to
604 affect the response of different trophic levels to changes in water availability and
605 will therefore influence the predicted outcomes of global change scenarios.

606 **Author contributions**

607 RW, AK, SJ and SH designed the study. RW generated and analysed the data. RW
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611

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617

618 **Data Accessibility**

619 All data are available in public archive Dryad. doi:10.5061/dryad.t6m9m

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872

873 **Supporting Information**

874 Additional supporting information may be found in the online version of this article.

875

876 Appendix S1 Supplementary Methods

877

878 Figure S1 Soil moisture measurements throughout a representative two weeks.

879

880 Figure S2 (a) Water content of barley plants treated with different herbivory and watering
881 regimes. (b) Stomatal conductance of barley plants treated with the different watering
882 regimes.

883

884 Table S1 Results of linear models, testing the effect of the treatments and their interactions on
885 different measures of aphid performance.

886

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Figures

Fig. 1. Total plant biomass (g dry mass (DM)) per pot of barley plants grown under controlled conditions treated with different watering regimes (ambient, drought and drought/deluge) with and without wireworm herbivory in (a) controlled environment and (b) field conditions. Values represent mean \pm standard error bars of 18 replicates for all watering regime and herbivory treatments apart from ambient watering regime without wireworms which represent 17 replicates for controlled environment, and 5 replicates for all watering regime and herbivory treatments for the field experiment. Bars sharing the same letter were not significantly different as determined by *Post-hoc* Tukey contrasts. Statistical analysis: (a) Controlled environment, watering regime $F_{2,93}=33.01$, $P<0.001$, wireworms $F_{1,93}=13.85$, $P<0.001$, watering regime x wireworms $F_{2,93}=3.93$, $P<0.05$. (b) Field mesocosm, watering regime $F_{2,20}=61.33$, $P<0.001$, wireworms $F_{1,20}=0.11$, $P>0.05$, watering regime x wireworms $F_{2,20}=0.03$, $P>0.05$

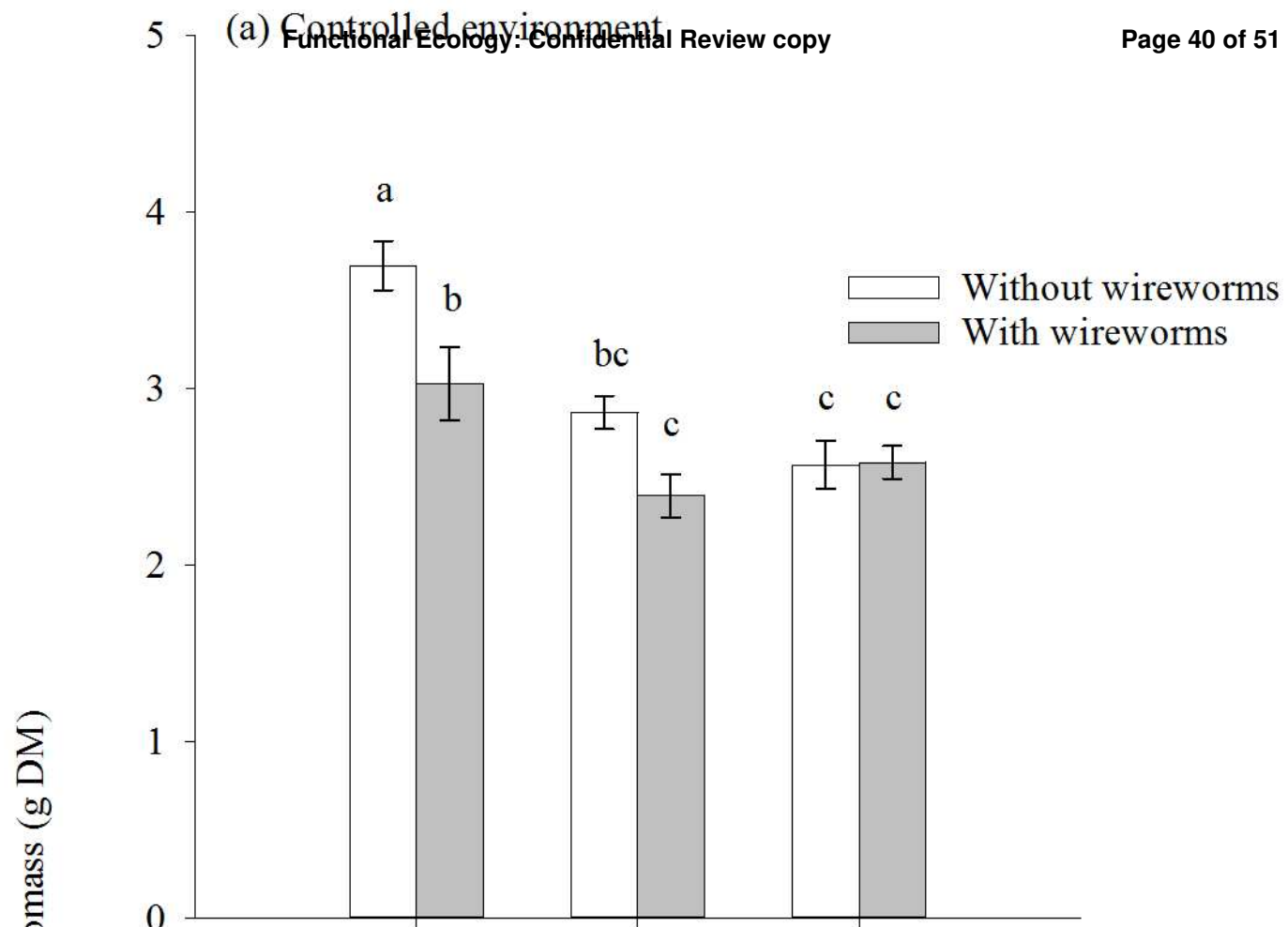
Fig. 2. Controlled environment: (a) Aphid mass (mg fresh mass (FM)) after 4 weeks of feeding on barley plants treated with three watering regimes (ambient, drought and drought/deluge). (b) Ladybird mass gain (mg fresh mass (FM)) after feeding on aphids collected from barley plants treated with ambient, drought and drought/deluge watering regimes. Values represent mean \pm standard error bars of 30 to 31 replicates for aphid mass and 11 to 13 replicates for ladybird mass gain. Bars sharing the same letter were not significantly different as determined by *Post-hoc* Tukey contrasts. Statistical analysis: (a) Aphid mass, watering regime $F_{2,39}=3.49$, $P<0.05$. (b) Ladybird mass, watering regime $F_{2,14}=3.78$, $P<0.05$

Fig. 3. Total number of aphids counted on three randomly selected barley plants per pot in field mesocosm with three different watering regimes in the presence or absence of wireworm herbivory over three weeks. Values represent mean \pm standard error bars of five replicates. Stars represent significant effect of wireworm herbivory on the number of aphids as determined by *Post-hoc* Tukey contrasts $P < 0.001^{***}$. Statistical analysis, watering regime $\chi^2 = 0.547$, $df = 2$, $P > 0.05$, wireworms $\chi^2 = 17.74$, $df = 1$, $P < 0.001$, week $\chi^2 = 105.92$, $df = 1$, $P < 0.001$, watering regime \times wireworms $\chi^2 = 27.45$, $df = 2$, $P < 0.001$, watering regime \times week $\chi^2 = 14.58$, $df = 4$, $P < 0.01$, wireworm \times week $\chi^2 = 15.22$, $df = 2$, $P < 0.001$, watering regime \times wireworm \times week $\chi^2 = 34.4307$, $df = 4$, $P < 0.001$.

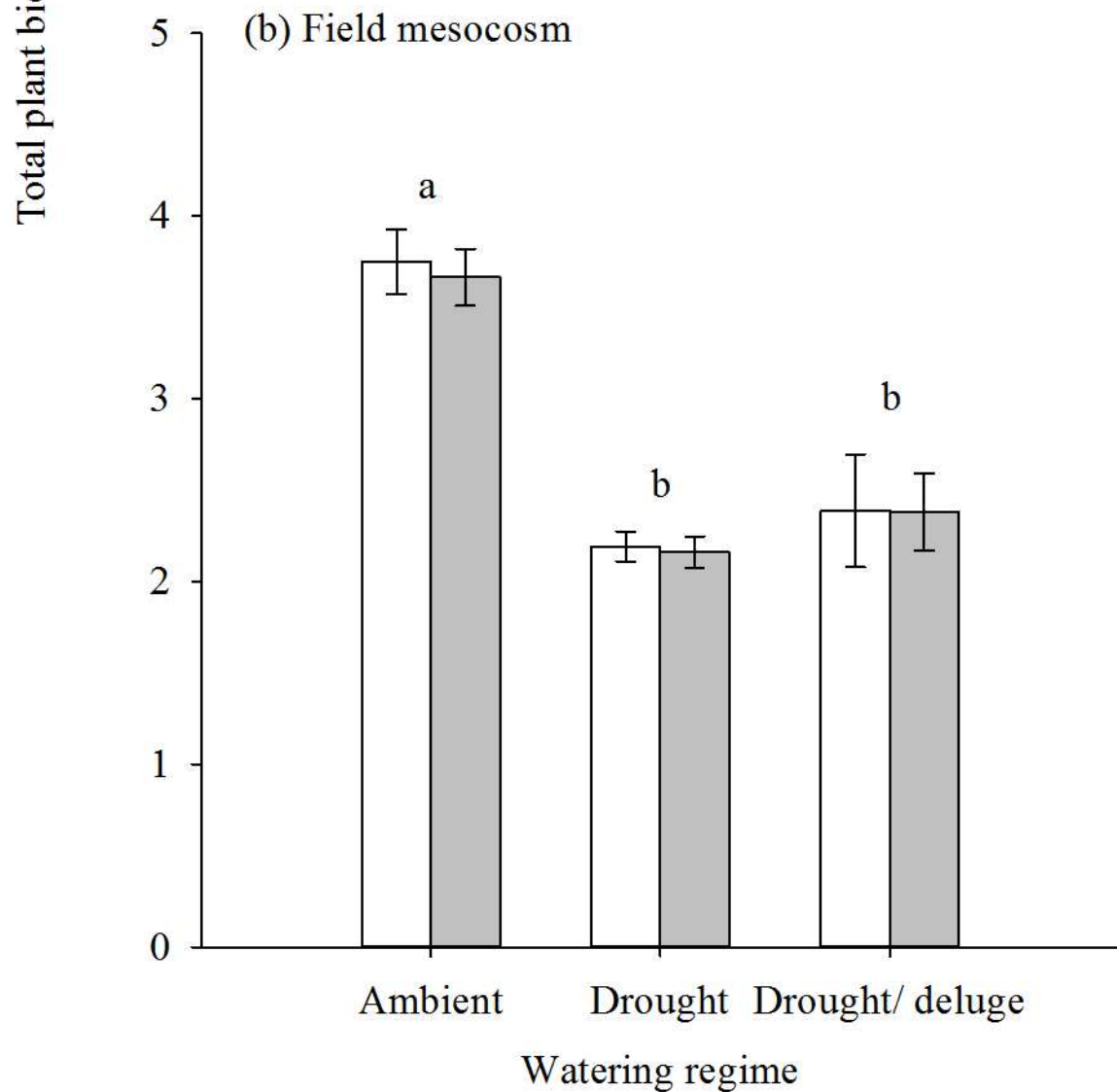
Fig. 4. Leaf concentrations of (a) Si (b) nitrogen (N), and (c) amino acids for barley plants grown under controlled conditions treated with different watering regimes (ambient, drought and drought/ deluge) and wireworm herbivory (b only). Values represent mean \pm standard error bars of 14 replicates (c) or 28 replicates (a and b). Bars sharing the same letter were not significantly different as determined by *Post-hoc* Tukey contrasts. Statistical analysis: (a) Leaf Si, watering regime $F_{2,74} = 68.22$, $P < 0.0001$. (b) Leaf N, watering regime $F_{2,74} = 12.78$, $P < 0.0001$, wireworm $F_{1,74} = 4.29$, $P < 0.05$. (c) Amino acids, watering regime $F_{2,90} = 3.36$, $P < 0.05$.

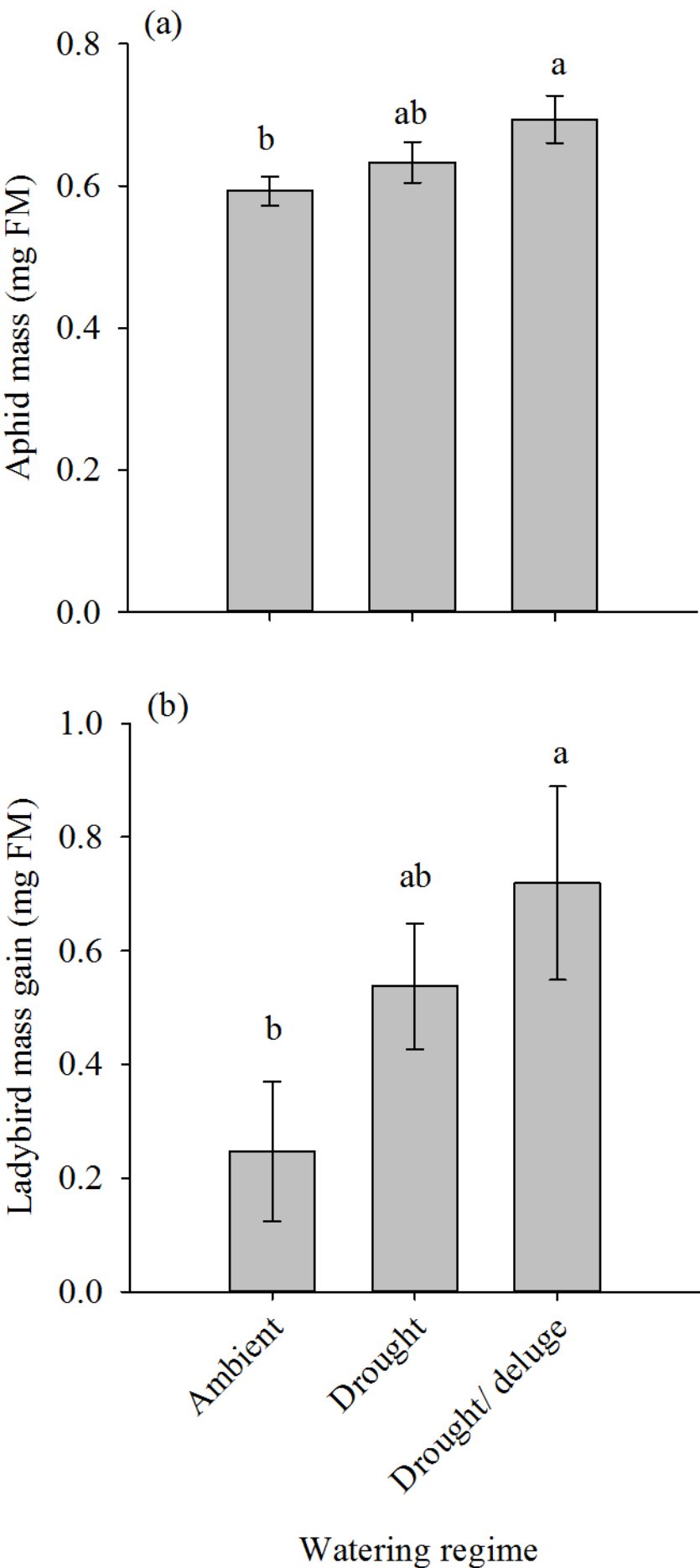
Fig. 5. Principal component analysis of amino acid mol% data in green leaf material sampled from plants grown under controlled conditions at harvest treated with different watering regimes (ambient, drought and drought/ deluge). (a) The mean sample scores plotted onto PC1 and PC2, which explain 68.8% and 22.0% of the variation in the data set, respectively. (b) Attribute loadings on the first two components PC1 and PC2. Standard abbreviations are:

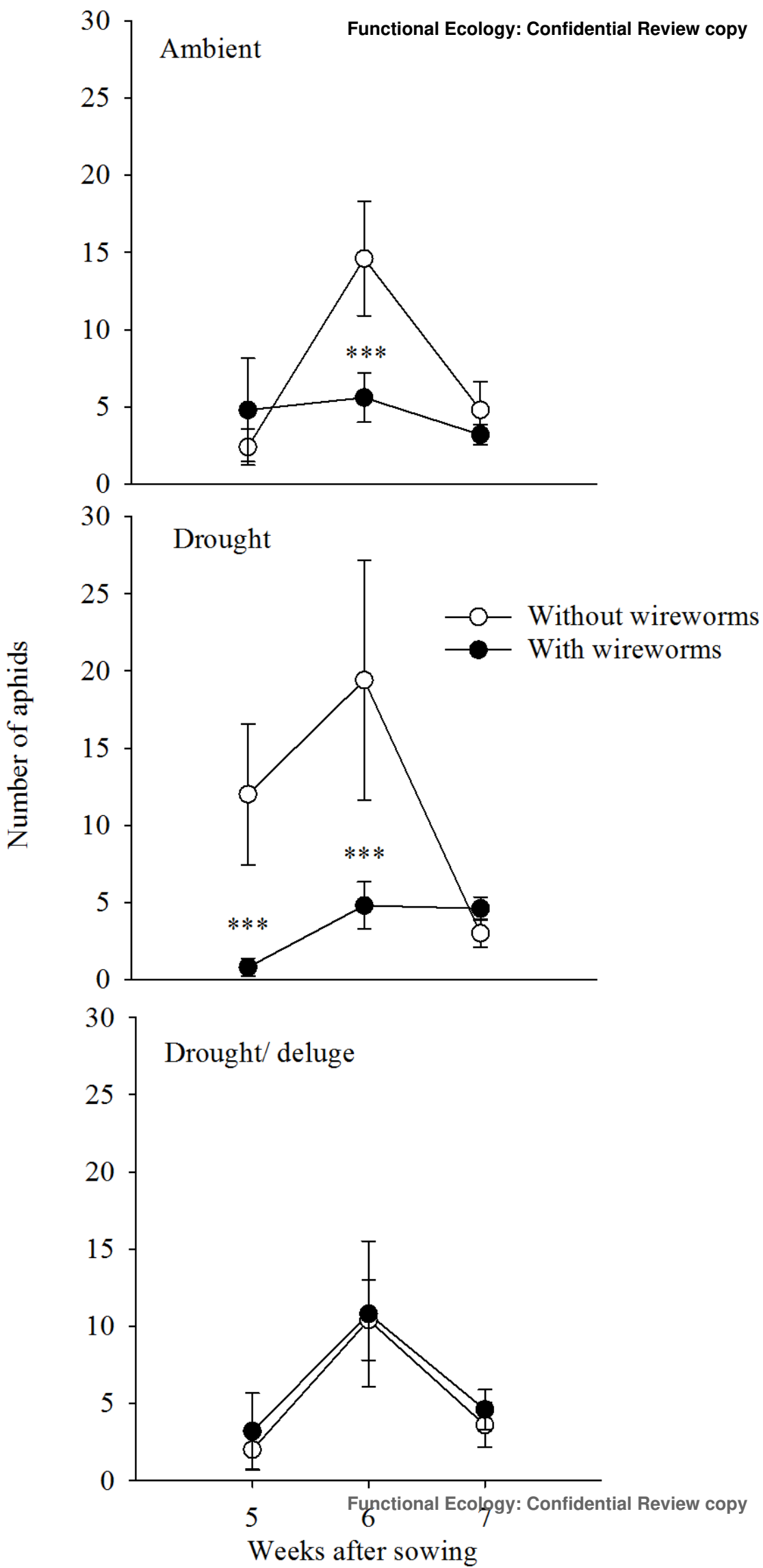
937 Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartate; Glu, glutamate; Gln, glutamine;
938 Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe,
939 phenylalanine; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

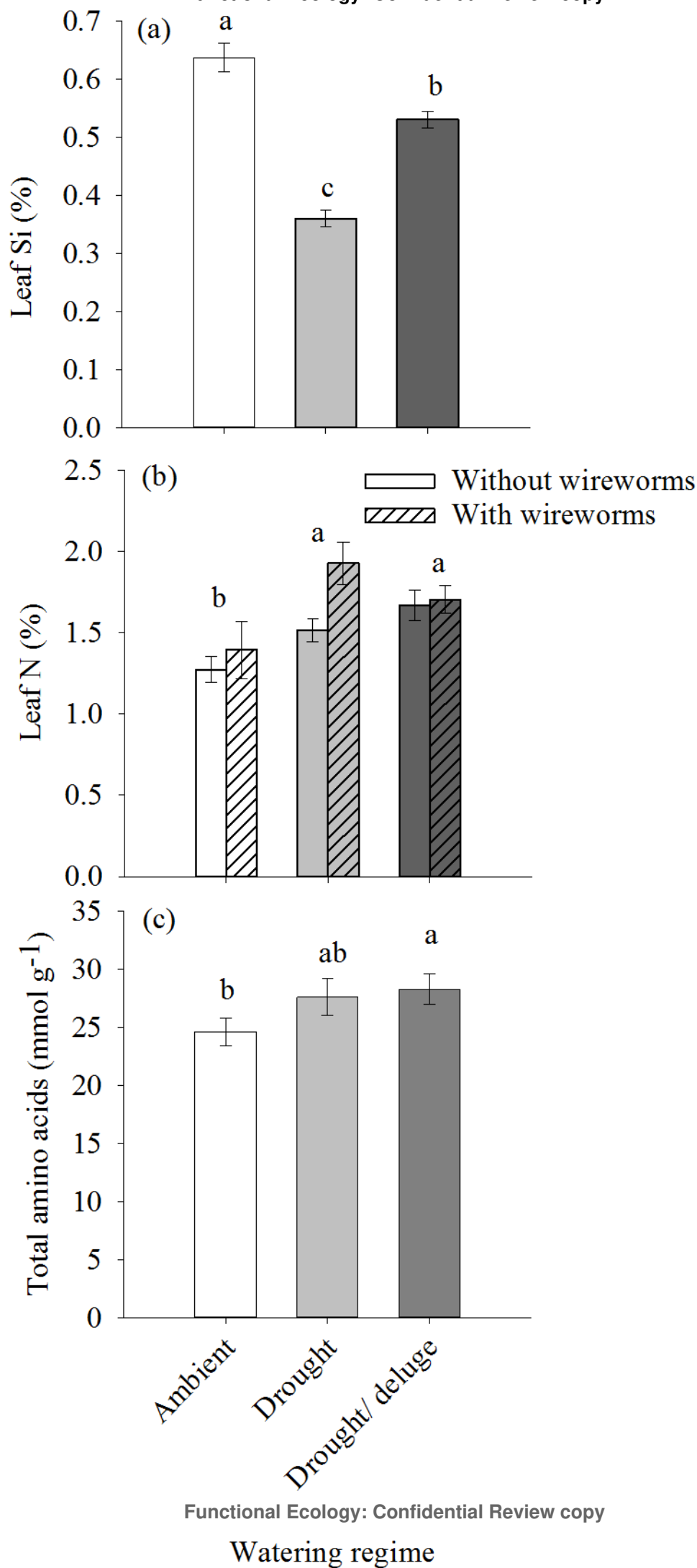


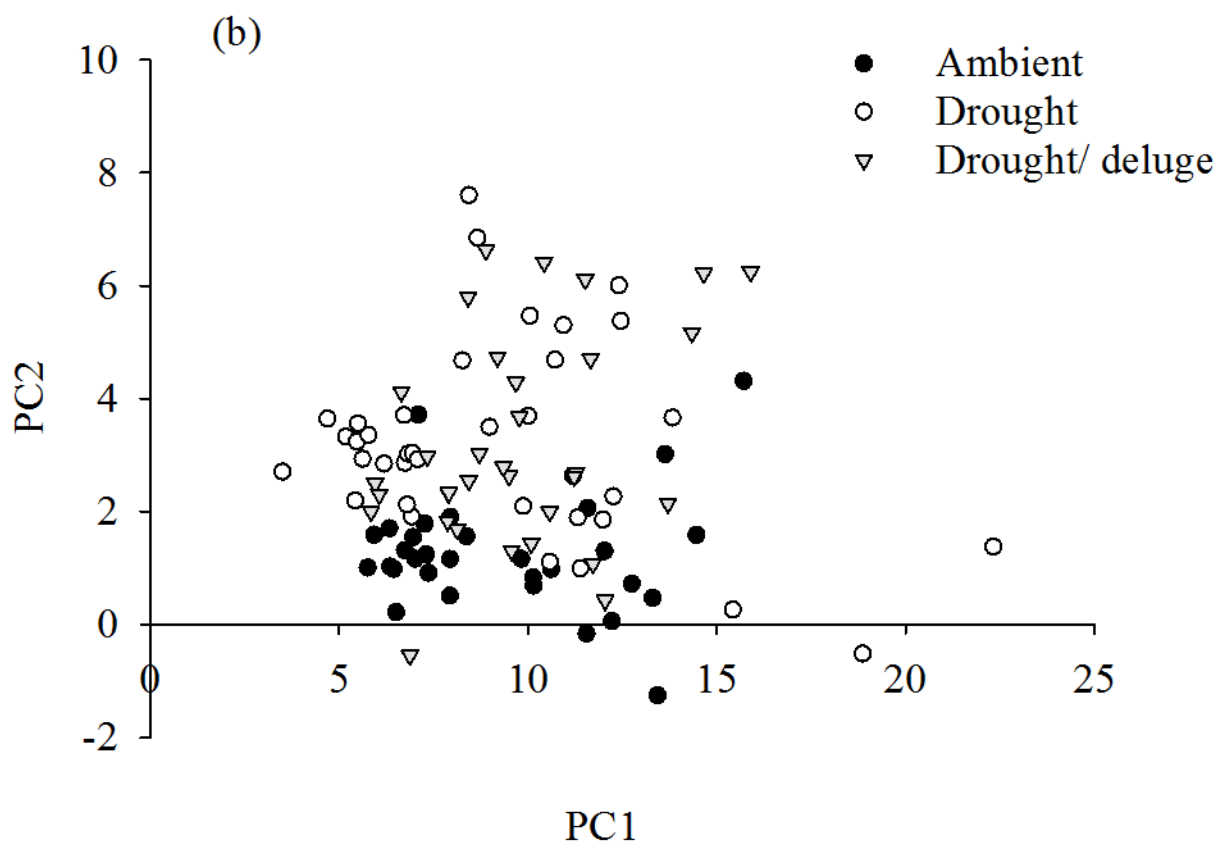
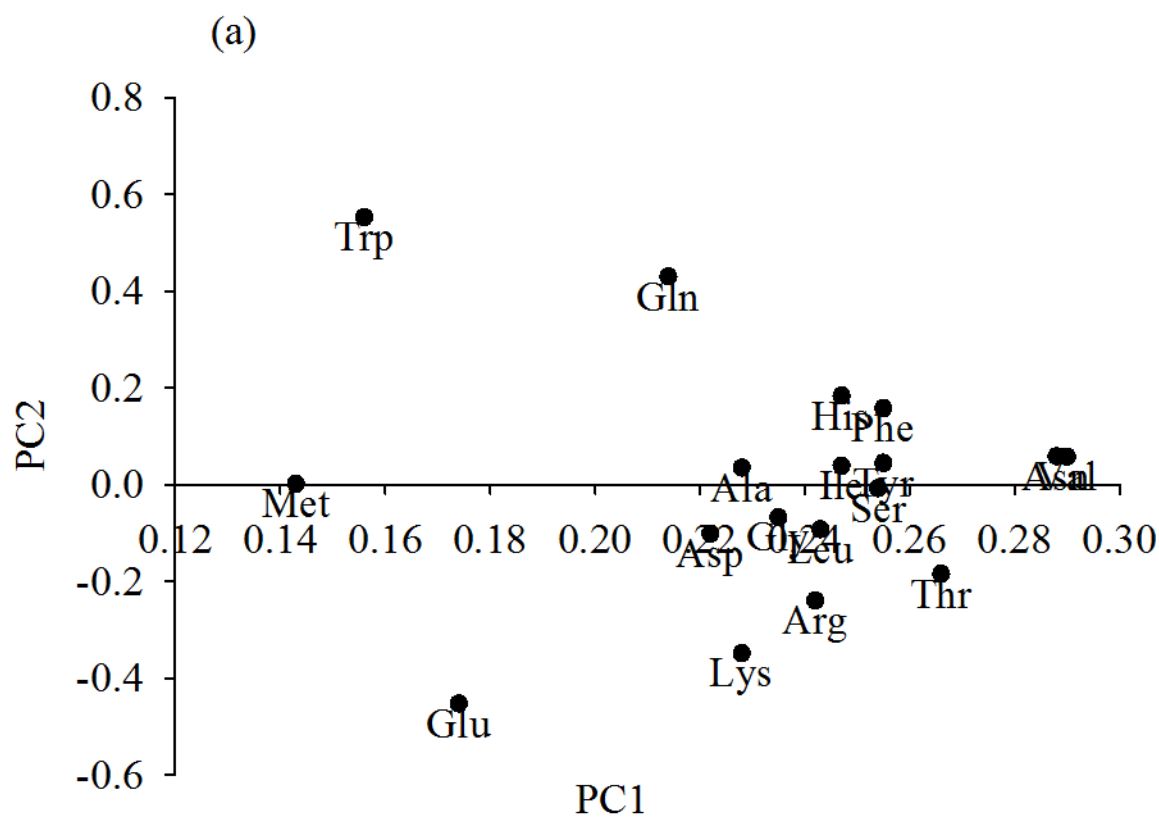
(b) Field mesocosm











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1 Appendix S1 Supplementary methods

2

3 *Insect culturing*

4 Prior to both experiments, wireworms were maintained on potato tubers (cv. Rooster; Albert
5 Bartlett, UK) at 7 °C (Johnson *et al.* 2008). For the controlled environment experiment,
6 aphids (*S. avenae*) were maintained on *H. vulgare* cv. Optic plants in a controlled
7 environment room at 15 °C and with 16/ 8 h day/ night. Four weeks after sowing, three adult
8 apterous aphids were caged (25 mm internal diameter clip cages suspended from metal
9 frames above the plant) to a fully expanded leaf on the main stem of plants assigned the
10 above-ground herbivory treatment. Cages without aphids were also placed on plants not
11 assigned the above- ground herbivory treatment. After 24 h, the adults and all but three
12 nymphs were removed from each cage (cage 1). After one week, all cages and aphids were
13 transferred to a fully expanded leaf of similar age on a tiller due to senescence of the original
14 leaf.

15

16 *Plant growth measurements and experimental design*

17 The controlled environment experiment comprised of a randomized block design with nine
18 blocks that were staggered temporally by two to three weeks to facilitate the final destructive
19 harvest. Plants were grown in 2.4 L pots with 18.5 cm diameter top of the pot and 13 cm
20 diameter at the bottom. Initially, two seeds were placed equidistant from the other seed and
21 the centre of each pot and buried c. 2 cm from the soil surface. To initiate germination, pots
22 were watered from the top twice per week for two weeks with 200 mL of deionised water.
23 Shortly after germination the number of seedlings was reduced to a single plant per pot of
24 consistent height and developmental stage. Plants were sufficiently spaced to allow this to
25 happen without disturbing neighbouring plants.

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26

27 For the field experiment, plants were grown under rain exclusion shelters which consisted of
28 a wooden frame supporting a 2100 mm × 3500 mm polycarbonate sheet (6 mm thick
29 polycarbonate sheeting, Polycarbonate Direct, Hull, UK) at an angle of 6° from a maximum
30 height of 1176 mm to the minimum height of 800 mm. Total area under the rain exclusion
31 shelter was 7.92 m². Initially, 18 pre-germinated seeds (soaked in deionised water at room
32 temperature for *c.* 24 h and incubated on Petri-dishes lined with damp paper towelling at 15
33 °C for 3 days) were sown randomly across the surface of the each pot *c.* 2 cm from the soil
34 surface on 24th June 2013. Pots were watered from the top twice per week for two weeks with
35 500 ml of deionised water. Following germination, seedling number was reduced to nine
36 plants per pot of consistent height and development stage randomly spread across the pot
37 surface to represent typical barley high sowing densities.

38

39 The saturation and desiccation (dried at 105 °C for 7 days) mass of the soil used in both
40 experiments was measured and from this the total water holding capacity was calculated.
41 When harvesting the plants, root: mass ratio was calculated by dividing the dry mass of roots
42 by total dry plant biomass.

43

44 *Plant chemical analysis*

45 Water content (g) of the plant material was calculated by subtracting the dry mass from the
46 fresh mass.

47

48 For silicon (Si) analysis, milled plant material was pressed at 11 tons into 5 mm thick
49 cylindrical pellets with a manual hydraulic press using a 13 mm die (Specac, Orpington, UK)

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50 prior to XRF analysis. C/N ratio was calculated using percentage C and N of the leaves from
51 flash combustion and chromatographic separation.

52

53 To analyse leaf amino acids for the controlled environment experiment, two green leaves
54 from each plant were removed from the leaf fraction before oven-drying and rapidly frozen in
55 liquid nitrogen for amino acid analysis. After 15 min extraction with gentle agitation (Bulker
56 shaker, MM 400, Retsch, Hope Valley, UK), samples were centrifuged first at 10 000 g for
57 15 min and the supernatant transferred to a clean tube. The remaining pellet was re-extracted
58 in 1 mL extraction solution following the same procedure. The pooled supernatant was
59 centrifuged at 15 000 g for 15 min to pellet any remaining leaf powder and aliquots of
60 supernatant were dried to a residue using a speedvac followed by freeze-drying (Freeze Dryer
61 Modulyo, Edwards, Apeldoorn, the Netherlands). β -Aminobutyric acid was used as an
62 internal control. Samples were stored at -20°C prior to analysis, when they were re-dissolved
63 in 1 mL ultra-pure water and aliquots (10 μ l) prior to reverse-phase HPLC analysis.

Functional Ecology

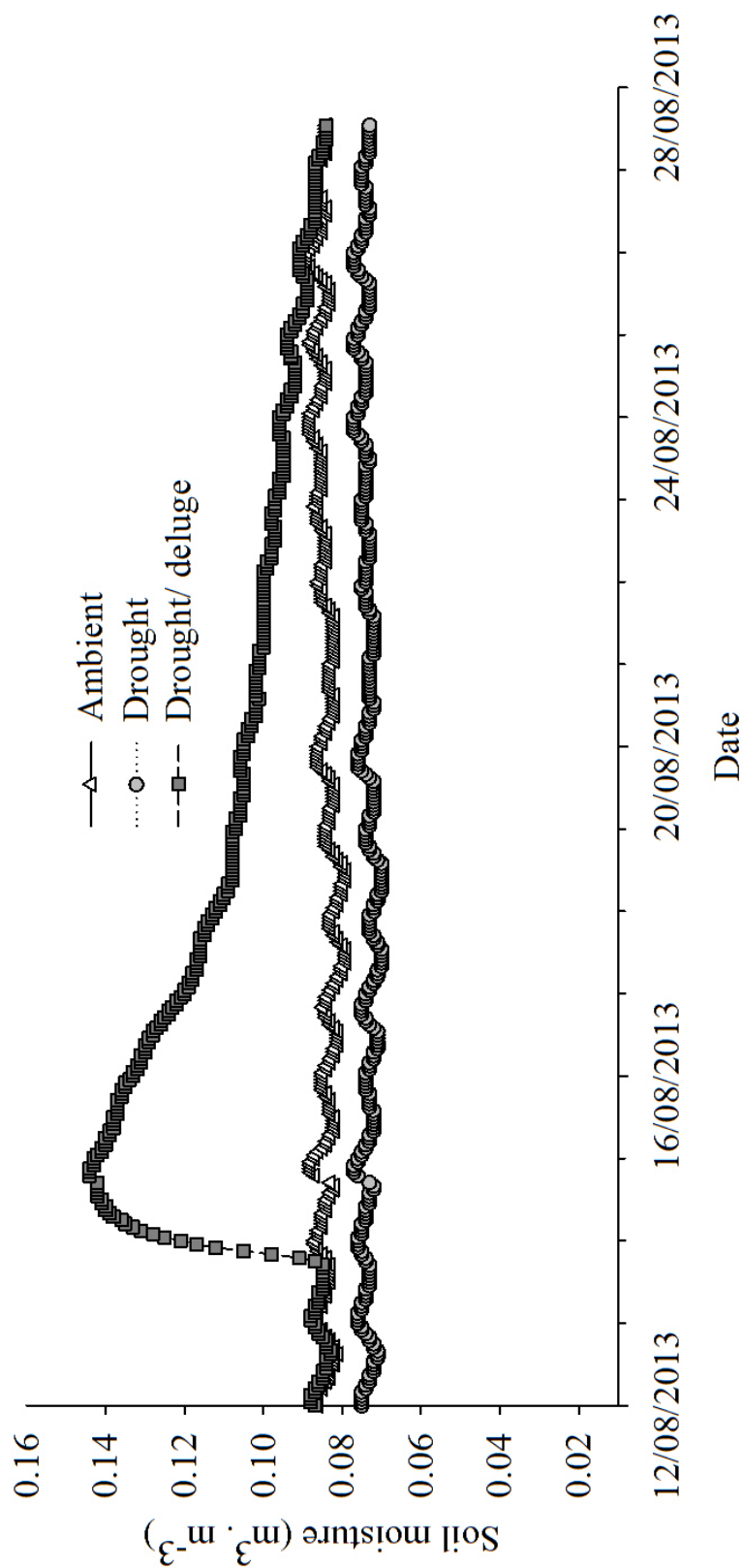
Table S1. Results of linear models showing F or χ^2 statistic, degrees of freedom (df) and p value (P) testing the effect of watering regime, wireworm herbivory and their interactions on different measures of aphid performance.

	Watering regime			Wireworm herbivory			Watering regime x wireworm herbivory		
	χ^2 or F	df	P	χ^2 or F	df	P	χ^2 or F	df	P
Number of days to aphid reproduction	$\chi^2=3.45$	2	1.77	$\chi^2=0.44$	1	0.51	$\chi^2=1.92$	2	0.38
Aphid fecundity	$\chi^2=1.17$	2	0.56	$\chi^2=0.30$	1	0.59	$\chi^2=0.94$	2	0.63
Aphid survival	F=0.93	2,39	0.40	F=0.00	1,39	1.00	F=1.45	2,39	0.25

1

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1 Figure S1. Soil moisture ($\text{m}^3 \cdot \text{m}^{-3}$) measurements in pots under different watering regimes
2 throughout a representative two weeks.



Functional Ecology

1 Figure S2. (a) Water content (g) of barley plants treated with different watering regimes,
2 ambient, drought and drought/ deluge with (hatched bars) and without aphid and wireworm
3 herbivory (grey bars). (b) Stomatal conductance of barley plants treated with different
4 watering regimes (ambient, drought and drought/ deluge). Values represent mean \pm standard
5 error bars of 9-8 replicates for water content and six replicated for stomatal conductance.
6 Bars sharing the same letter were not significantly different as determined by *Post-hoc* Tukey
7 contrasts. Statistical analysis, (a) watering regime $F_{2,95}=55.23$, $P<0.001$, wireworm
8 $F_{1,95}=6.46$, $P<0.001$, (b) watering regime $F_{2,64}=29.41$, $P<0.001$.

